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A STUDY OF UPTAKE OF RADIOLABELED HOST PROTEINS AND PROTEIN SYNTHESIS DURING DEVELOPMENT OF EGGS OF THE ENDOPARASITOID, *MICROPLITIS CROCEIPES* (CRESSON) (BRACONIDAE)

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Abstract—The uptake of radiolabeled haemolymph and fat body proteins from fourth instar larvae of *Heliothis zea* (Boddie) by eggs of *Microplitis croceipes* (Cresson) was examined by SDS–polyacrylamide gel electrophoresis and by autoradiography. None of the ^{125}I-labeled haemolymph proteins was detected in eggs exposed to the proteins *in vivo*. Although several of the proteins were observed in eggs incubated with the labeled proteins *in vitro*, none of these proteins was degraded or resynthesized into new structural proteins during development of the embryo. Similarly, no significant uptake of labeled fat body proteins by the eggs could be detected *in vitro*. On the other hand, protein synthesis measured by incorporation of [^{35}S]methionine occurred throughout egg development. Proteins were synthesized at least 1 hr after the egg was deposited into the host. The protein patterns of eggs on one-dimensional SDS gels were complex and ranged in size from less than 18,500 to more than 330,000 mol. wt. The protein band patterns of the newly synthesized proteins showed some qualitative differences at 1–8, 16–32 and 40 hr after egg deposition. We conclude that eggs do not absorb or utilize the host apoproteins (or degradation products) but instead synthesize proteins *de novo* from free amino acids in the host haemolymph.

Key Word Index: All of title, haemolymph, SDS–polyacrylamide gel electrophoresis, fat body, growth, embryogenesis

INTRODUCTION

It is generally assumed that eggs of endoparasitic Hymenoptera absorb haemolymph or components of the haemolymph through the egg membranes (Fisher, 1971). This view likely came about from the observation that most eggs of parasitoids were thin-walled, contained little yolk when laid and increased in volume many times after they were first deposited (Flanders, 1942; Simmon, 1947; Jackson, 1928; Smith, 1952; Ivanova-Kasas, 1972). The embryonic membranes or trophamnion around the eggs of polyembryonic species has been suggested as the reason for this large increase in volume before eclosion (Doutt, 1947). At eclosion the trophamnion cells are liberated into the haemolymph of the host and have been suggested to have a trophic or protection function (Vinson and Iwantsch, 1980). Actual evidence that the maturing egg indeed takes up nutrients is, however, lacking. The following study was made to determine whether the eggs of the braconid *Microplitis croceipes* (Cresson) absorb the host's haemolymph proteins for subsequent growth of the embryo, or whether protein synthesis occurs throughout egg development as has been demonstrated in *Drosophila* embryos (Roberts and Gragiosi, 1977; Savoini *et al.*, 1981; Lockshin, 1966; Santon and Pellegrini, 1981) and in the housefly *Musca domestica* L. (Pretruschka and Bier, 1972).

MATERIALS AND METHODS

Host and parasitoid colony maintenance

The host species, *Heliothis zea* (Boddie), was mass reared on a pinto bean based diet in individual 28.3 g cups at 25°C, 55% r.h. and 14 hr light:10 hr dark photoregime (Leppla *et al.*, 1982, 1984).

The parasitoid *M. croceipes* was reared in the laboratory at 23°C with 60–70% r.h. and a 14 hr light:10 hr dark photoperiod. The adults were held in Plexiglas® (acrylic) cages (25 × 25 × 25 cm, with a sock entrance). Water that contained chloramphenicol (1 mg/ml) was provided in glass vials containing dental wicks and food was provided by streaking undiluted honey on the inside of the cages. Late second and third instar host larvae were exposed to adult wasps (5 wasps: 15 hosts) for 1 hr in plastic petri dishes (100 × 15 mm) under fluorescent lighting. The wasps were then removed to the holding cages and the parasitized larvae were placed individually into plastic cups to prevent cannabalism. Each cup contained approx. 7.1 g of diet. The larvae were allowed to develop for 7 days at which time most of the diet was removed to facilitate collection of the wasp pupae 7 days later. These pupae were then placed in the Plexiglas cages containing food and water where the adult wasps emerged.

To obtain larger numbers of eggs (more than one egg per host) for experimental use, hosts were exposed to a higher parasitoid to host ratio (16–24 wasps: 8 hosts). This resulted in up to 15 parasitoid eggs per host.

Measurement of egg volume

The volume of the parasitoid egg was estimated by using the formula for the volume of a cylinder: $V = \pi r^2 l$, where r = radius and l = length in mm. After dissection from the host, the eggs were held in a buffer solution with an osmolarity comparable to the host's haemolymph [0.358 osmolar, 0.05 M Tris–HCl, pH 7.5, 0.3 M sucrose and 0.01% 1-phenyl-2-thiourea (PTU)] to prevent melanization. The dimensions of the eggs were measured under a dissecting scope with an ocular grid.

Collection of haemolymph for ^{125}I-labeling

Haemolymph was collected from early fourth instar larvae of *H. zea* that weighed 52–125 mg (< 16 hr after molt). Haemolymph was released by clipping a proleg and was collected in a 20 μl glass capillary tube. It was diluted to 50% v/v with 0.05 M Tris–HCl, pH 8.0, that contained 0.01% PTU in a 0.4 ml polyethylene centrifuge tube. Samples were centrifuged in a Beckman® microfuge at 9380 ***g*** for 5 min to remove cellular material and stored at −69°C.

^{125}I-labeling of host haemolymph proteins

Two methods of radioiodination were used to label the haemolymph of *H. zea*. The IODO-GEN® method (Fraker and Speck, 1978), which employs a mild oxidant, did not result in efficient labeling of the proteins. The chloramine-T method (Fidge, 1974; Fidge and Poulis, 1974), which utilizes a stronger oxidizing agent, resulted in labeled proteins that could be observed in autoradiographs and was subsequently used. Initially, 100 μg of *H. zea* haemolymph protein in 112 μl of 0.05 M Tris–HCl, pH 7.5, that contained 0.01% PTU was added to each of four disposable glass tubes. One millicurie of ^{125}I (13.3 mCi/μg ^{125}I sp. act.; Amersham) was added to each tube and 100 μl of chloramine-T (4 mg/ml) in 0.05 M phosphate buffer, pH 7.5 was added to start the reaction. At 30, 60, 150 and 300 sec after addition of the chloramine-T, 200 μl of sodium meta-bisulfite (2.4 mg/ml) was added to stop the reaction. In labeling subsequent batches of the haemolymph, a 90-sec incubation time was used. Each sample solution was dialyzed against 3.8 l Ringer's solution (Humason, 1961) for 6 hr at 4°C and then dialyzed against fresh Ringer's overnight. This dialysis procedure was found sufficient to remove all unbound ^{125}I. The samples were counted in a Packard Autogamma® counter (Model 5130) and stored at −69°C.

In vivo *labeling experiments*

^{125}I-labeled haemolymph was injected into early fourth instar *H. zea* larvae (20 hr after molt from third to fourth instar) that were first exposed to *M. croceipes* females (3 females/host) for 1 hr. Immediately after being stung, each larva was injected with 1.5–2.0 μg of protein (5.5×10^5–1.0×10^6 cpm) in 6–7 μl of Ringer's solution delivered by a 33-gauge needle and an ISCO microapplicator (Model M®). The dorsal intersegmental membrane (between metathorax and abdomen) of 18 larvae were injected. The larvae were then placed on individual pinto bean diets in 28.3 g plastic cups and held at 26°C with 50% r.h. At 4, 12, 24, 36 and 48 hr after injection, haemolymph was collected and prepared as described above.

Collection of ^{125}I-labeled eggs

After collection of the haemolymph, each larva was dissected and the parasitoid eggs were removed and rinsed 3 times with 0.05 M Tris–HCl, pH 7.5 containing 0.4 M sucrose and 0.01% PTU. The eggs were then placed in additional buffer at 2 eggs/μl and sonicated for 60 sec at full power at 0°C in a 375-W cuphorn sonicator (Model W-375, Heat Systems-Ultrasonics, Inc.). The solution containing egg contents and egg chorions was centrifuged in the Beckman® microfuge for 5 min. The supernatant was saved and the egg pellets were rinsed, resuspended, and centrifuged twice. The resultant pellet was resuspended in 20 μl of buffer. The samples were stored at −69°C.

Incubation of eggs with ^{125}I-labeled proteins

Eggs of *M. croceipes* were dissected from host *H. zea* larvae at 0, 8 and 24 hr after the larvae were stung. The eggs were then transferred to Goodwin's IPL 52B medium (minus yeastolate; Goodwin and Adams, 1980) that contained ^{125}I-labeled proteins from control and parasitized larvae. Generally, 15–35 eggs were placed in 200 μl medium and 50 μl of Ringer's solution that contained 1 μCi of ^{125}I-labeled control or parasitized haemolymph (9×10^3 cpm/μl medium) was added and incubated at 28°C for 17 hr. After incubation, the eggs were combined to give a total of 200 for each time period and were treated as above for the eggs labeled with ^{125}I *in vivo*.

^{35}S-labeling of fat body proteins in vitro

Fat body was dissected from surface-sterilized larve (70% ethanol) into Goodwin's IPL-52B medium (minus yeastolate and methionine) purchased from K C Biological. After five rinsings (5 times with fresh medium) the net wet weight was determined and 5–7 mg of the tissue was incubated in 300 μl of medium plus 1 μl of an aqueous solution that contained 1 μCi [^{35}S]methionine (303.8 Ci/mmol; Amersham) for 17 hr. The medium containing labeled proteins released by the fat body was stored at −69°C until several batches were collected, pooled, dialyzed and concentrated against Ringer's solution using a ProDiMen® (10,000 mol. wt cut off membrane) apparatus.

Incubation of eggs with ^{35}S-labeled fat body proteins

Eggs of *M. croceipes* were dissected from host *H. zea* larvae and transferred to Goodwin's IPL-52B medium (minus yeastolate) that contained the concentrated fat body-conditioned medium fraction with the released labeled proteins (4 parts medium: 1 part fat body concentrate). Generally, samples of 33–99 eggs were placed into 400 μl medium and 100 μl fat body-conditioned medium concentrate which contained 4.3×10^5–7.5×10^5 cpm of labeled protein (80 cpm/μl medium). After incubation at 28°C for 17 hr, the eggs were removed and treated as above for the collection of eggs labeled with ^{125}I-labeled proteins *in vivo*.

Measurement of protein synthesis in parasitoid eggs in vitro

After early fourth instar larvae (20 hr after molt) of *H. zea* were exposed to females of *M. croceipes* for 1 hr, they were held in individual plastic cups that contained pinto bean diet at 25°C and 50% r.h. At 8-hr intervals larvae were dissected and parasitoid eggs were collected. Eggs were rinsed 3 times with tissue culture medium IPL-52B and incubated with either [^{3}H]leucine (sp. act. 1109 mCi/mmol) or [^{35}S]methionine (sp. act. 430 mCi/mmol) for 2 hr at 23°C and 58% r.h. The eggs were then rinsed with 10% trichloroacetic acid (TCA) 3 times and homogenized in 150 μl 10% TCA; finally they were sonicated for 120 sec (as described above). The homogenate was then filtered with a glass fiber filter (Gelman AE, 25 mm) and unincorporated isotope was removed by washing with 25 ml 10% TCA. The fiber filter was then counted in 10 ml of Insta-Gel® scintillation fluid (New England Nuclear). Samples were counted in a Packard (2450) Tricarb® liquid scintillation counter (79% counting efficieny).

SDS electrophoresis and autoradiography

Haemolymph samples labeled with either ^{125}I or [^{35}S]methionine were separated by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) according to Swanton *et al.* (1975). Briefly, the samples were heated for 3 min at 100°C in 1 mM dithiothreitol, 1% SDS and 5% glycerol. After electrophoresis, gels were stained with a 1% (w/v) solution of Coomassie brilliant blue® R-250 in 50% (w/v) trichloroacetic acid for 20 min then destained overnight with methanol–acetic acid–water (5:1:5, by vol). Polyacrylamide gel electrophoresis (PAGE) was done according to Davis (1964). Gels used for autoradiography were dried onto filter

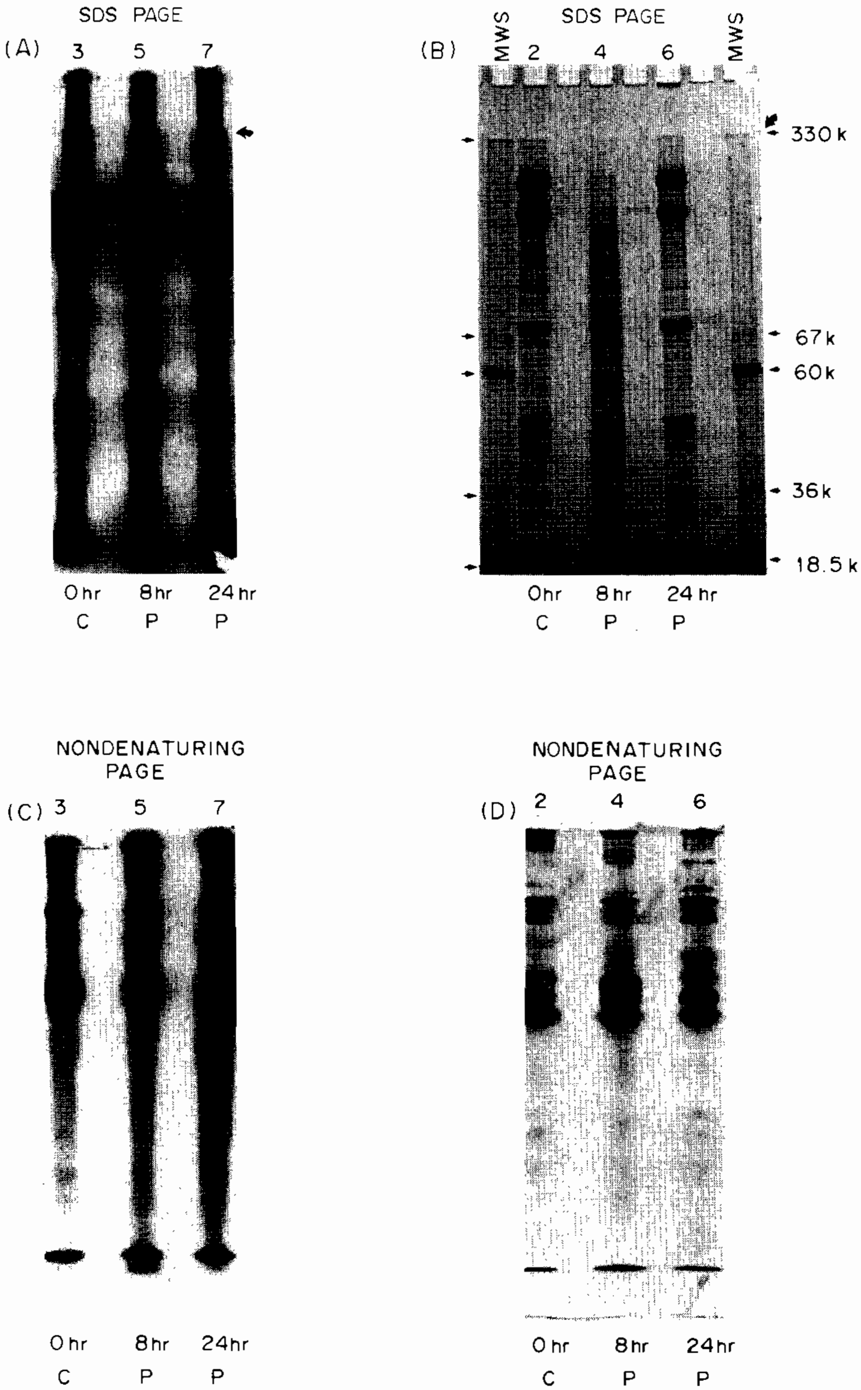

Fig. 1. Comparison of unlabeled haemolymph from fourth instar *H. zea* larvae on a SDS polyacrylamide gel, 12.5%: (A) autoradiograph, (B) Coomassie Brilliant Blue stain of gel in A. Non-SDS polyacrylamide gel, 7.5%: (C) autoradiograph, (D) Coomassie Brilliant Blue stain of gel in C. To both the SDS gel and non-SDS gel, 100 and 70 μg of non-labeled haemolymph protein were applied per lane and 7×10^4 cpm of label was applied per lane. Molecular weight standards (MWS*) were applied to the SDS gel only. Gel lanes: (1) MWS* (30 μg); (2) 0-hr control haemolymph; (3) 0-hr ^{125}I-labeled control haemolymph; (4) 8-hr parasitized haemolymph; (5) 8-hr ^{125}I-labeled parasitized haemolymph; (6) 24-hr parasitized haemolymph; (7) 24-hr ^{125}I parasitized haemolymph. *MWS: thyroglobulin *b* (3.3×10^5), albumin (6.7×10^4), catalase (6×10^4), lactate dehydrogenase (2×10^4) and ferritin (1.85×10^4). Broad arrows indicate the surface of the separating gel.

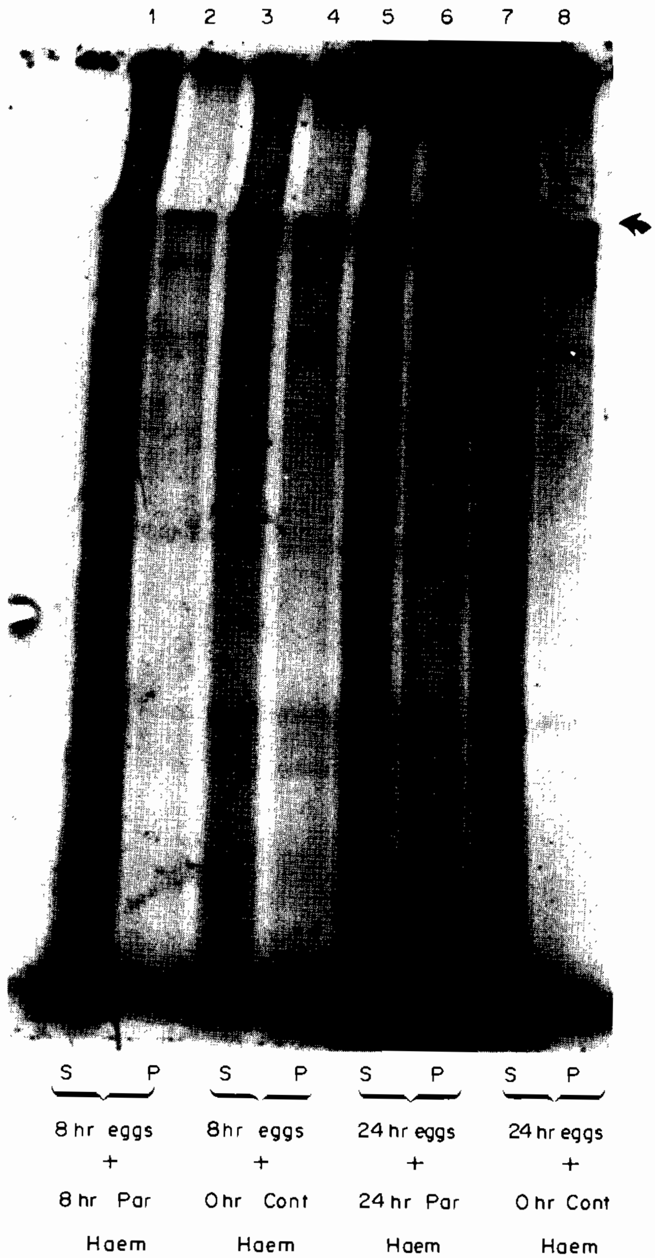

Fig. 2. Comparison of uptake of radiolabeled proteins by eggs of *M. croceipes* from media containing control vs parasitized *H. zea* haemolymph. Autoradiograph of eggs that were incubated with ^{125}I-labeled haemolymph for 17 hr, rinsed well with buffer, and then applied to a 12.5% SDS polyacrylamide gel. Gel lanes: (1) supernatant (9.4×10^3 cpm); (2) pellet (7.8×10^3 cpm) from 8 hr eggs plus 8 hr parasitized haemolymph; (3) supernatant (6.2×10^3 cpm); (4) pellet (1.2×10^4 cpm) from 8-hr eggs plus 0-hr control haemolymph; (5) supernatant (2.4×10^4 cpm); (6) pellet (7.2×10^4 cpm) from 24-hr eggs plus 24-hr parasitized haemolymph; (7) supernatant (1.7×10^4 cpm); (8) pellet (1.1×10^4 cpm) from 24-hr eggs plus 0-hr control haemolymph. Arrow indicates surface of separating gel.

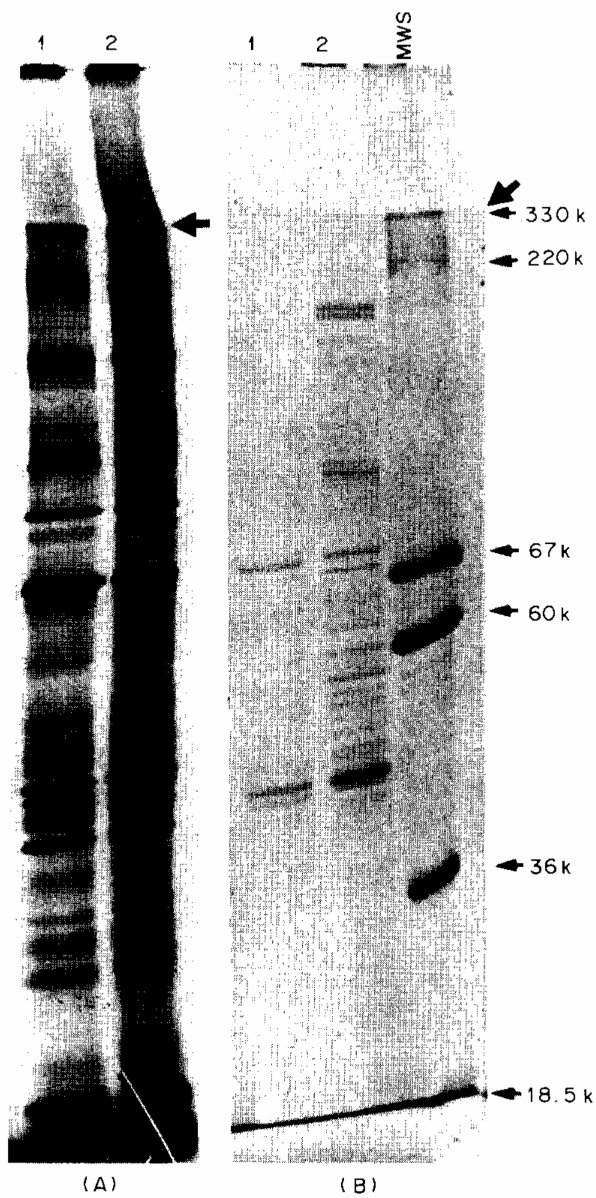

Fig. 3.(A) Autoradiograph of [^{35}S]methionine-labeled proteins released by *H. zea* fat body *in vitro* and applied to a SDS polyacrylamide gel, gel lanes: (1) 5×10^4 cpm; (2) 7.4×10^4 cpm applied per lane. (B) Same as in A but stained with Commassie Brilliant Blue. Molecular weight standards (MWS, 30 μg); thyroglobulin *b* (3.3×10^5), ferritin (2.2×10^5), albumin (6.7×10^4), catalase (6×10^4), lactate dehydrogenase (2×10^4) and ferritin (1.85×10^4). Broad arrows indicate surface of separating gel.

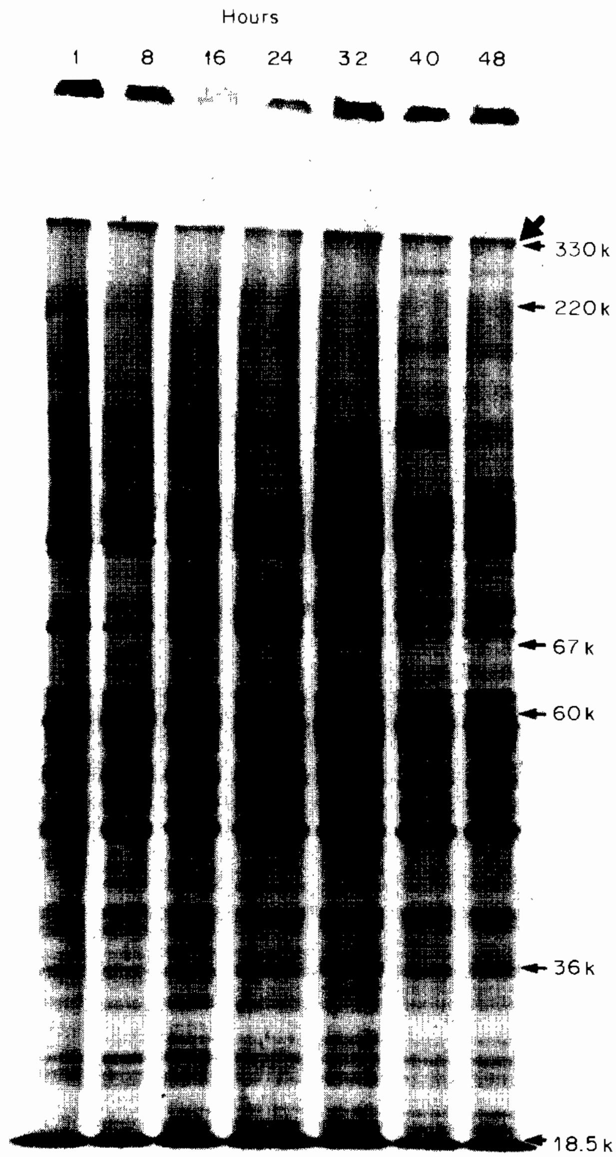

Fig. 5. Protein synthesis by eggs of *M. croceipes* dissected from *H. zea* host larvae at various times after deposition and incubated with [^{35}S]methionine *in vitro*. Autoradiograph of egg supernatants separated on 12.5% SDS polyacrylamide gels; 86,000 cpm of each egg supernatant were applied per lane. Molecular weight standards were run in the outside lanes and stained with Coomassie Brilliant Blue. Molecular weight standards (MWS, 15 μg): thyroglobulin (3.3×10^5), ferritin (2.2×10^5), albumin (6.7×10^4), catalase (6×10^4), lactate dehydrogenase (3.6×10^4) and ferritin (1.85×10^4) at the bromophenol blue front. Broad arrows indicate surface of the separating gel.

paper *in vacuo* at 70 C. Dried gels were exposed to LKB Ultrafilm ^{3}H® at -70 C for various periods of time. Film was developed with KLX Rapid Developer and Fixer® (Kodak). To improve detection of ^{125}I-labeled proteins, dried gels were sandwiched between two intensifying screens (Cronex®, Dupont).

RESULTS

^{125}I-*labeling of proteins*

Haemolymph of *H. zea* radiolabeled with ^{125}I using the chloramine-T method was initially examined by SDS–PAGE and PAGE. In the autoradiograph of the SDS gel (Fig. 1A), band patterns of the parasitized haemolymph at 8 and 24 hr were similar to the 0-hr control haemolymph. In Coomassie Brilliant Blue stained gels (Fig. 1B), two heavily stained, high molecular weight bands that were evident in the 0-hr control and 24-hr parasitized haemolymph stained lightly in the 8-hr parasitized haemolymph, but showed up as heavily labeled bands in the autoradiograph (Fig. 1A). In two previous electrophoretic runs of 8-hr parasitized haemolymph, these high molecular weight bands appeared heavily stained with Coomassie Brilliant Blue. Thus, the autoradiographic experiments provided more consistent data than this Coomassie Brilliant Blue staining. Figure 1 (C and D) shows that iodination of the proteins did not alter their mobility after PAGE when compared with unlabeled haemolymph stained with Coomassie Brilliant Blue.

In vivo *experiments*

When aliquots of the ^{125}I-labeled proteins were injected into host larvae, very little label was subsequently found associated with the eggs, even though radioactivity was still present in the circulating haemolymph 17 hr after the proteins were injected (Table 1). The amount of label associated with newly hatched first instar larvae at 36 hr (experiments B and C) also was low.

No radioactive bands were evident when the total label in the egg supernatants and pellets were combined for each sample, run on SDS gels, and analyzed on autoradiographs, apparently because of the low quantity of radioactivity associated with the samples.

In vitro *experiments*

Incubation of parasitoid eggs with ^{125}*I-labeled haemolymph proteins.* Electrophoretic separation of *M. croceipes* egg proteins from eggs incubated with ^{125}I-labeled *H. zea* haemolymph proteins showed that only several labeled haemolymph proteins were resolved in the egg supernatant and even fewer in the egg pellet (Fig. 2). There was no distinct difference between eggs incubated in parasitized haemolymph versus control haemolymph. Nor was there any difference between 8 and 24-hr eggs incubated in the two labeled haemolymphs. Additionally, there did not appear to be any degradation and resynthesis of the labeled haemolymph proteins into any new egg proteins, since the labeled proteins evident in the supernatant and pellet of the eggs had mobilities similar to those in the electrophoretic pattern for labeled haemolymph alone (Fig. 1A).

Incubation of parasitized eggs with [^{35}*S]methionine labeled fat body proteins* in vitro. Figure 3 shows the electrophoretic separation of [^{35}S]methionine labeled proteins released into the culture medium by host fat body over a 17-hr incubation period. When eggs of *M. croceipes* were incubated in the medium for 17 hr, the cpm accumulated per egg were too low to be detected in autoradiographs (Table 2). Also, there were no significant differences between uptake of labeled proteins by eggs at 8 or 24 hr after being deposited in the host or uptake of label by eggs that were incubated with proteins derived from fat body from parasitized versus control hosts.

Table 1. Uptake of ^{125}I label by *M. croceipes* eggs *in vivo**

Post-sting and injection (hr)	cpm injected/larva	cpm μl haemolymph	Number of parasitoid eggs	Total cpm in eggs (n)†	Total cpm in egg supernatant	Total cpm in egg pellet	Mean ($\pm$SE) (cpm/egg)
4	6.7×10^5	5408	37	490 (2)	230	260	13.5 ± 3.5
12	8.5×10^5	7526	27	1487 (3)	1068	419	54.7 ± 43.7
24	7.5×10^5	5887	25	563 (3)	312	250	6.7 ± 6.7
36	8.8×10^5	2582	12‡	1231‡ (2)	965‡	267‡	$101.5‡ \pm 42.5$
48	1.0×10^6	3341	21‡	1617‡ (1)	598‡	1019‡	77.0‡

*Eggs removed at indicated times after oviposition from *H. zea* larvae injected with ^{125}I-labeled haemolymph proteins immediately after being stung.
†Number of replications in parentheses.
‡First instar parasitoid larvae.

Table 2. Uptake of [^{35}S]methionine labeled fat body proteins by eggs of *M. croceipes in vitro*

Post-sting* (hr)	cpm in medium	Total number eggs	Total cpm	Mean ($\pm$SE) (cpm/egg)†
Parasitized host fat body proteins				
8	6.3×10^5	170	3404	19.3 ± 1.5
24	6.0×10^5	217	5770	25.7 ± 7.0
Control host fat body proteins				
8	6.0×10^5	184	5810	26.5 ± 14.3
24	5.3×10^5	229	2680	12.0 ± 1.0

*Mock post-sting in controls.
†Three replicates.

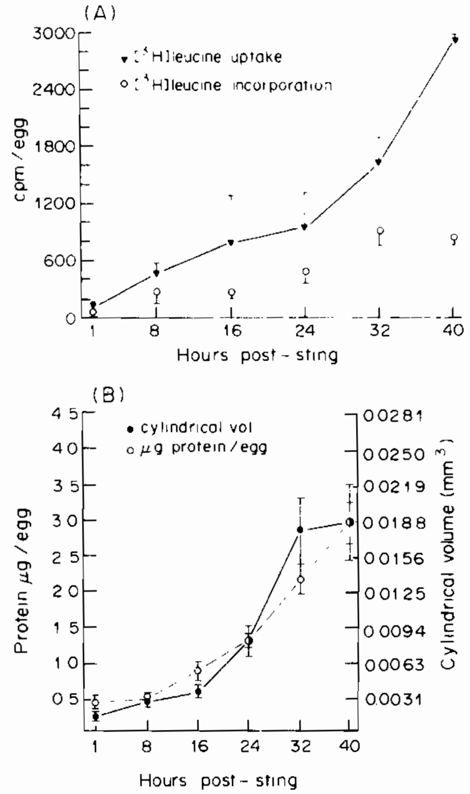

Fig. 4.(A) Protein synthesis, and (B) increase in total protein and egg volume of eggs of *M. croceipes* dissected from *H. zea* larvae at various times after egg deposition.

Protein synthesis

Figure 4A shows protein synthesis was detectable 1 hr after the parasitoid egg was deposited then increased gradually and leveled off at 32 hr (4 hr prior to hatching). The amount of free [^{3}H]leucine taken up by the eggs was significantly greater than that incorporated into protein. Total protein and the cylindrical volume of the egg followed a similar pattern, exhibiting a steep increase at 16–40 hr (Fig. 4B).

The electrophoretic pattern of proteins synthesized during development of the parasitoid egg is shown in Fig. 5. It is evident that the embryo is engaged in synthesizing proteins as early as 1 hr after the egg is desposited. Several other interesting facts emerged from this experiment. One is that the pattern of protein synthesis is complex at all stages, the newly synthesized proteins ranging in size from less than 18,500 to at least more than 330,000 mol. wt (comparing Fig. 5A with molecular weight standards in Fig. 5B). Second, the patterns of proteins synthesized at different times of development were similar. However, some differences were evident in certain areas. The band patterns at 1 and 8 hr were qualitatively similar to each other but were different in certain areas (>67,000 mol. wt) from the 16–32 hr band patterns. The band pattern for first instar larvae at 48 hr was similar to that of eggs 40 hr after deposition.

DISCUSSION

In most free-living insects, oöcyte growth depends upon uptake of yolk precursor proteins or vitellogenins from the haemolymph. Uptake of vitellogenin involves the formation of pinocytotic vesicles in the external membrane of the oöcytes and is taken into the oöcyte (Engelmann, 1979). It is generally assumed that the eggs of endoparasitoids absorb nutrients and other materials necessary for growth and development from the host's haemolymph (Fisher, 1971). This poses the question of whether the parasitoid egg takes up whole or partially degraded proteins from the host's haemolymph.

We did not observe uptake of ^{125}I-labeled host proteins by the parasitoid eggs *in vivo*. In contrast, several labeled bands were resolved in autoradiographs of eggs exposed to label proteins *in vitro*; however, they did not appear to be incorporated into new egg proteins. This difference between the results obtained *in vivo* and those obtained *in vitro* was probably due to the generally higher concentration of ^{125}I-proteins the eggs were exposed to *in vitro*.

The host haemolymph proteins labeled by the chloramine-T method and observed in autoradiographs on non-SDS gels ran similar to unlabeled proteins detected with Coomassie Brilliant Blue, indicating the total net charge and molecular weight of the proteins had not been altered by the iodination. However, it is possible that the ^{125}I-proteins could still be recognized as different from the native haemolymph proteins by the egg. To further examine this possibility, uptake of [^{35}S]methionine labeled proteins synthesized by the host's fat body was studied. The fat body, a cellular tissue in close association with the haemolymph, is recognized as the biosynthetic source of plasma proteins (Wyatt and Pan, 1978). In this case some radioactivity was found in the eggs; however, not enough was present to be detected in autoradiographs of the parasitoid egg proteins. On this basis we concluded that the eggs of *M. croceipes* take up little or none of the host's haemolymph proteins. This does not preclude the possibility that certain haemolymph proteins have a role in the growth and development of the parasitoid. Transport proteins could release lipoidal materials to the egg for its absorption and utilization. Plasma proteins such as lipoproteins are important in transport of lipids and the transport of hormones such as juvenile hormones by proteins in the haemolymph of insects is well documented (Wyatt and Pan, 1978).

Because the results of the ^{125}I and ^{35}S-labeling experiments indicated that the parasitoid eggs did not significantly take up the host's haemolymph proteins, we decided to examine protein synthesis in the eggs during their growth and development. The occurrence of protein synthesis as early as 1 hr after deposition of the eggs into the host and the variety of polypeptides synthesized at that time and throughout development of the egg indicated that host haemolymph proteins, at least the apoproteins, are not needed for growth by *M. croceipes* and that the parasitoid egg relies on its own protein synthetic capabilities for growth. Whether the initiation of synthesis of these proteins depends on fertilization or deposition of the egg into the host, or whether it is a continuation of the synthesis of proteins, which were initially synthesized prior to egg deposition, remains to be determined.

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